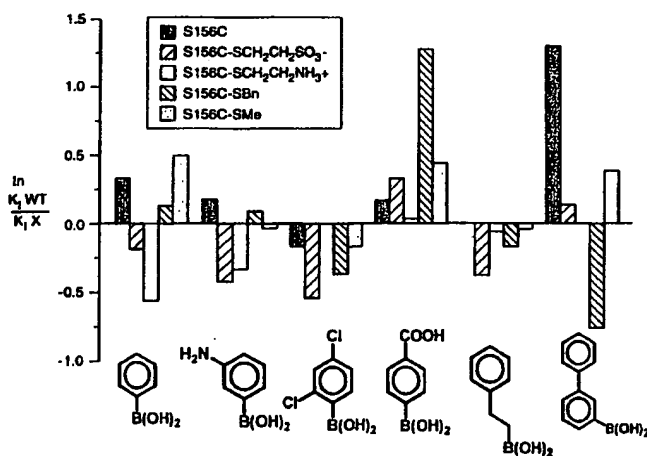




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicants: GENENCOR INTERNATIONAL, INC. [US/US]; 4 Cambridge Place, 1870 South Winton Road, Rochester, NY 14618 (US). JONES, J., Bryan [CA/CA]; 1275 Seaforth Crescent, Lakefield, Ontario K0L 2H0 (CA).		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
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(54) Title: CHEMICALLY MODIFIED ENZYMES



(57) Abstract

Modified enzymes are provided in which at least one amino acid, such as asparagine, leucine, methionine or serine, of an enzyme is replaced with a cysteine and the thiol hydrogen is replaced with a substituent group providing a thiol side chain selected from the group consisting of: a) $-SR^1R^2$, wherein R^1 is an alkyl and R^2 is a charged or polar moiety; b) $-SR^3$, wherein R^3 is a substituted or unsubstituted phenyl; c) $-SR^4$, wherein R^4 is substituted or unsubstituted cyclohexyl; d) $-SR^5$, wherein R^5 is $C_{10}-C_{15}$ alkyl; and e) $-SR^6$ wherein R^6 is a C_{1-6} alkyl. Also, methods of producing the modified enzymes are provided, as well as detergent and feed additives and a composition for the treatment of a textile. A method for using the modified enzymes in organic synthesis is additionally provided. Further, modified enzymes having improved activity, altered pH profile and/or wash performance are provided.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/21446

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N9/00 C12N9/54 C11D3/386

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BERGLUND, PER ET AL: "Altering the specificity of subtilisin B. lentus by combining site-directed mutagenesis and chemical modification" BIOORG. MED. CHEM. LETT. (1996), 6(21), 2507-2512 CODEN: BMCLE8; ISSN: 0960-894X, 6 November 1996, XP002063093 see the whole document	1-9, 15-24
Y	---	1,10-16, 25-42
Y	WO 91 16423 A (NOVONORDISK AS) 31 October 1991 cited in the application see the whole document ---	1,15,16, 30-33, 37-42
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

5 August 1998

Date of mailing of the international search report

28.08.98

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Van der Schaal, C

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 97/21446

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BECH L AND BREDDAM K: "Chemical modification of a cysteinyl residue introduced in the binding site of carboxypeptidase Y by site-directed mutagenesis" CARLSBERG RESEARCH COMMUNICATIONS, vol. 53, 1988, pages 381-393, XP002063095	1-4,7,9, 13,14, 16-19, 22,24, 28,29
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Y	--- WYNN R AND RICHARDS F: "Chemical modification of protein thiols: formation of mixed disulfides" METHODS IN ENZYMOLOGY, vol. 251, 1995, pages 351-356, XP002063094 see the whole document	1,15,16, 30
Y	--- WO 96 27671 A (GENENTECH INC) 12 September 1996 see the whole document	10-12, 25-27, 31-37, 39,40
Y	--- EP 0 328 229 A (GIST BROCADES NV) 16 August 1989 see the whole document	31-33, 38,41,42
Y	--- BONNEAU P ET AL: "Alteration of the specificity of subtilisin BPN' by site directed mutagenesis..." JOURNAL OF THE AMERICAN CHEMICAL SOCIETY., vol. 113, 1991, pages 1026-1030, XP002073658 DC US cited in the application see the whole document	38,41,42
Y	--- GLOSS L AND KIRSCH J: "Examining the structural and chemical flexibility of the active site base, Lys-258, of Escherichia coli aspartate aminotransferase by replacement with unnatural amino acids" BIOCHEMISTRY, vol. 34, 1995, pages 12323-12332, XP002063096 see abstract	1,15,16, 30
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INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 97/21446

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>BERGLUND, PER ET AL: "Chemical modification of cysteine mutants of subtilisin Bacillus lentus can create better catalysts than the wild-type enzyme"</p> <p>J. AM. CHEM. SOC. (1997), 119(22), 5265-5266 CODEN: JACSAT;ISSN: 0002-7863, 4 June 1997, XP002063097</p> <p>see the whole document</p> <p>-----</p>	1-42

INTERNATIONAL SEARCH REPORT

Inter. application No.
PCT/US 97/21446

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 2-6, 17-21 34 completely, 1, 7-12 16 22-27 31-33 partially

Modified enzyme wherein one or more amino acid residues are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR1R2 in which R1 is an alkyl and R2 is a charged or polar moiety.

2. Claims: 35 completely, 1 7-14 16 22-29 31-33 partially

Modified enzyme wherein one or more amino acid residues are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR3 in which R3 is a substituted or unsubstituted phenyl

3. Claims: 36 completely 1 7-14 16 22-29 31-33 partially

Modified enzyme wherein one or more amino acid residues are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR4 in which R4 is substituted or unsubstituted cyclohexyl

4. Claims: 37 38 completely 1 7-12 16 22-27 31-33 partially

Modified enzyme wherein one or more amino acid residues are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR5 in which R5 is C10-15 alkyl

5. Claims: 15 30 39-42 completely 31-33 partially

Modified enzyme wherein one or more amino acid residues selected from asparagine, leucine or serine are replaced by cysteine residues, wherein the cysteine residues are modified by replacing the thiol hydrogen with a substituent group of the general formula -SR6 in which R6 is C1-6 alkyl.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internati Application No

PCT/US 97/21446

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